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2) Kolodner and Marsischky, Curr Opin. Genet. Dev., 9(1):89-96 (Feb. 1999).
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Thanks in advance,

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Mismatch repair goes meiotic

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Until recently, geneticists studying mammalian meiosis have experienced what might best be described as 'system envy'. While studies of yeast and *Drosophila* have yielded remarkable advances in understanding how homologous recombination and meiotic chromosome pairing, synapsis and segregation occur in these organisms[1], the pace has been much slower for mammals. However, as exemplified by the paper by Baker *et al.*[2] on page 336 in this issue, the situation is beginning to change. The identification of mammalian homologues of non-mammalian meiotic genes, and the subsequent generation of mouse knockouts for these genes, now make it possible to study the effects of specific mutations on the meiotic process.

Baker *et al.*[2] have used this approach to study one of the murine DNA mismatch repair genes, *Mlh1*. At first glance, this might seem an unlikely target. The mouse Mlh1 protein is homologous to Mut1, one of the components of the MutHLS mismatch repair system of *E. coli*[3]. This is a complex system, requiring products from several genes, but three genes are key: *mut5* (encoding a DNA mismatch-binding protein), *mutH* (encoding a synthesized-strand binding protein) and *mutL* (encoding a protein that appears to promote MutS–MutH interactions and to enhance MutH activity). As the primary role of this system is the repair of replication errors it is not surprising that several of its components have been conserved through evolution; thus, genes encoding homologues to *MutL* and *MutS* have been identified in mammals as well as yeast.

In humans, mismatch repair mutants recently have attracted considerable attention due to their association with malignancy[4]. Mutations in either *MSH2* (homologous to *mutS*) or in *PMS1*, *PMS2* or *MLH1* (homologues to *mutL*) occur in families segregating hereditary non-polyposis colon cancer, one of the common cancer predisposition syndromes. Similarly, mice engineered to carry mutations at mismatch repair loci have an increased susceptibility to tumour formation[5].

However, in addition to its role in somatic tissues, mismatch repair fulfills an important function in the germ cell during meiotic prophase. A central feature of meiosis I is homologous recombination, an important component of which is the recognition and repair of mismatches in the heteroduplex DNA. Abnormalities in the repair system can have catastrophic meiotic consequences, as evidenced by studies in *Saccharomyces cerevisiae*. Mutations in the mismatch repair gene homologues *MSH2*, *MSH4*, *MSH5* and *PMS1* have been linked to a variety of meiotic abnormalities, including aberrant recombination, post-meiotic segregation and nondisjunction[6].

Baker *et al.*[2] demonstrate that mutations in mammalian mismatch repair also disrupt the meiotic process. They used homologous recombination to target the murine *Mlh1* locus in embryonic stem cells and subsequently generated animals heterozygous and homozygous for mutation. The somatic effects were as expected: *Mlh1–/–* animals exhibit microsatellite instability and are likely predisposed to cancer, since a lymphoma was identified in a female sacrificed for ovarian studies.

The meiotic consequences are profound. Histological examination of testes for *Mlh1–/–* males demonstrate the presence of primary spermatocytes, but a complete absence of later stage cells, consistent with an arrest at meiosis I. Ovaries from *Mlh1–/–* females contain only a few follicles but corpea lutea are occasionally identified, indicating that ovulation can occur. However, breeding with *Mlh1–/–* females was unsuccessful and, as the males have no sperm, infertility is apparently a feature of both sexes.

What is the basis for these effects? To address this question, Baker *et al.*[2] took two approaches. First, they examined chromosome behaviour in meiosis I of *Mlh1–/–* spermatocytes. Initial pairing behaviour appears normal. However, by diplotene — when homologous chromosomes are normally still joined at the points of genetic exchange (the chiasmata) but are no

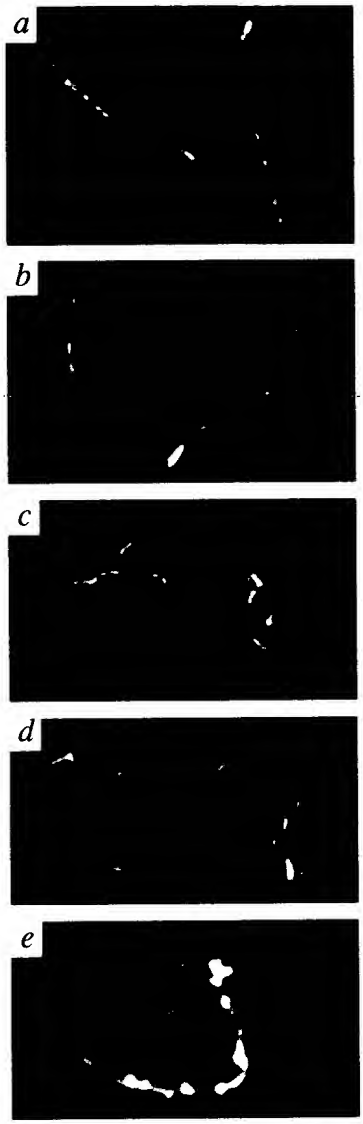

Fig. 1 Localization of Mlh1 during meiosis (white = anti-Rad51; red = anti-Mlh1). *a*, Pachytene oocyte: Mlh1 is seen as discrete foci along the paired axes of the synaptonemal complex. *b*, Early diplotene oocyte: As homologues begin to separate, Mlh1 is located at junctures between synapsed and desynapsed axes, as well as at sites along the still synapsed axes. *c*, Fiplotene oocyte: Mlh1 is still visible at chiasmata sites. *d*, Late diplotene oocyte: Mlh1 has disappeared from the chiasmata sites. *e*, XY chromosomes in early pachytene spermatocyte: a single Mlh1 focus is found at the base of the XY-pairing site.

Table 1 Effects of mismatch repair mutations on murine meiosis

Protein (ref.)	*E. coli* homologue	Effect on male meiosis	Effect on female meiosis
Mlh1 (2)	*MutL*	Reduced numbers of chiasmata, premature separation of bivalents; males infertile	Reduced numbers of chiasmata, premature separation of bivalents; females infertile
Pms2 (7)	*MutL*	synaptic abnormalities; males infertile	not tested; females fertile
Msh2 (5,7)	*MutS*	Meiotic studies not conducted; mice fertile	

longer fully synapsed — the homologous chromosomes in *Mlh1*-deficient spermatocytes are frequently separated from one another. That is, they are univalents. By metaphase of meiosis I, most of the chromosomes are in this configuration. This suggests that *Mlh1* promotes chiasma formation or stablilization and, consistent with this interpretation, analyses of diplotene and metaphase figures indicated a 10–100-fold reduction on chiasma frequency in *Mlh1–/–* spermatocytes.

Second, Baker *et al.*[2] used an antibody against Mlh1 to examine its meiosis I localization in deficient males, and in normal males and females (Fig. 1). In oocytes of normal females, discrete *Mlh1* loci observed on synapsed portions of the synaptonemal complex (SC) as early as zygotene. By early pachytene — at which time homologues are fully synapsed — several Mlh1 loci per SC are seen (Fig. 1*a*); by mid-pachytene the number drops to about 1.5 foci per SC; and by early diplotene few foci are left but occasionally are observed at the sites of chiasmata (Fig. 1*b–d*).

In spermatocytes, Mlh1 foci are not identified until early pachytene, but by mid-pachytene approximately 1.2 foci per SC are observed. Importantly, a focus is consistently identified at the distal end of the pairing region of the XY bivalent in early pachytene spermatocytes (Fig. 1*e*) but shortly thereafter — about the time when the X and Y begin to desynapse — no signal is observed.

These observations fulfill many of the predictions for a protein involved in chiasma processing: in *Mlh1–/–* spermatocytes, with a diminished number of chiasmata, there was no detectable Mlh1; in normal males and females, the total number of mid-pachytene foci corresponded to the expected number of chiasmata and more foci were identified in oocytes than spermatocytes, consistent with the known difference in female to male recombination rates; and, in females, foci were occasionally observed at the sites of crossing-over. Taken together, these results provide compelling evidence that Mlh1 fulfills an important function in meiosis I.

However, many important questions remain. For example, when does Mlh1 act and what is its target? Based on the temporal expression pattern of Mlh1, Baker *et al.*[2] speculate that it may be a component of late recombination nodules, electron dense structures which correlate with crossing-over in many organisms[1]. However, the nature and organization of mammalian recombination nodules remain obscure; thus, this association remains to be elucidated.

Also unclear are the meiosis I interactions, if any, between *Mlh1* and other mammalian mismatch repair proteins (see Table 1). In a previous study, Baker *et al.*[7] analysed the consequences to murine spermatogenesis of a *Pms2* null mutation. Pms2 and Mlh1 are thought to interact with one another in somatic tissues, as they appear to be components of same repair pathway and form a heterodimer *in vitro*[8]. Thus, it is not surprising that *Pms2* mutations also disrupt spermatogenesis and that *Pms2*-deficient males are sterile[7]. However, what is somewhat surprising is the difference in phenotype between the two mutations: Pms2 deficiency is evident earlier in meiosis I, associated with abnormalities in synapsis, and, unlike Mlh1 deficiency, *Pms2–/–* mice produce some, although abnormal, spermatozoa. Further, *Pms2*-deficient females are fertile, indicating gender-specific differences in the meiotic roles of Mlh1 and Pms2. Thus, it seems unlikely that Mlh1 and Pms2 function coordinately in meiosis I, nor do they appear to interact with the MutS mammalian homologue Msh2. *Msh2*-deficient mice are fertile[5,9], providing little evidence for a meiotic role of this protein.

Finally, the clinical implications of these findings are yet to be determined. The consequences of meiotic abnormalities to human reproduction are enormous. For example, an estimated 5% of all clinically recognized human pregnancies (that is pregnancies surviving to at least 6–8 weeks gestation) and possibly as many as 20–25% of all human conceptuses are aneuploid as a result meiotic chromosome mis-segregation. Further, these values increase with age of the women, so that among women 40 years and older, it may be that a majority of oocytes are chromosomally abnormal. Despite this high incidence and obvious clinical importance, the basis of these abnormalities remains a complete mystery. Could it be that some proportion are due to meiotic mutations or to age-related degradation in MLH1, PMS2 or in other meiotic proteins with which they interact? Further analyses of chromosome segregation, possibly using non-null mutations, will be extremely welcome.

Future studies notwithstanding, the present paper on *Mlh1* (ref. 2) and its predecessor on *Pms2* (ref. 7) are important milestones in mammalian meiotic analysis. This is not to say that mammalian geneticists do not still have ample reason to be envious of their colleagues studying meiosis in other organisms. They do. Yeast and *Drosophila* geneticists — with rough maps of the meiotic *highway* already in hand[1] — remain miles ahead of their mammalian counterparts. Nevertheless — at long last — mammalian geneticists can see the on-ramp.

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